

Evolutionary Gene Variation and Conservation in Land Race Cereals

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EVOLUTIONARY GENE VARIATION AND CONSERVATION
IN LAND RACE CEREALS

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- IV. BEKELE, E. 1983. The neutralist-selectionist debate and estimates of allozyme multilocus structure in conservation genetics of the primitive land races of Ethiopian barley. - *Hereditas* 99: 73-88
- V. BEKELE, E. 1984. Analysis of regional patterns of phenotypic diversity in the Ethiopian tetraploid and hexaploid wheats. - *Hereditas* 100: 131-154
- VI. BEKELE, E. 1984. Relations between morphological variance, gene diversity and flavonoid patterns in the land race populations of Ethiopian barley. - *Hereditas* 100: 271-294
- VII. BEKELE, E. 1984. The interface of genetics ecology and biosystematics in conservation: A dialogue. (in press - in ACTA UNIV. UPS. - SYMB. BOT. UPS.)

References to these papers will be made by the above Roman numerals.

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Introduction

Wherever we look in life, whichever dimension we use, we find uniqueness. And that uniqueness spells diversity. This is an accepted fact that goes back in the history of man himself (Mayr 1982). Although population geneticists claim to quantify biological diversity, there are many difficulties they face. This is because both creation of diversity and sorting of diversity are highly and hierarchically ordered in a way that can not be easily uncovered by the present techniques and methodologies.

Riedl (1978), using system theory, explains why the "natural system" is in hierarchic order, why the system of gene interactions shows the same pattern, and how the genome acquires the rational-seeming organization. Conrad (1983) gives an elaborate and detailed mathematical treatment using the laws of thermodynamics to explain the relationship of diversity and order in biological adaptability and reliability. Mayr's (1969) attempt to explain the integrity of the gene pool of the species is a round-about way to explain the biological order of diversity. Legendre (1971) also tried to analyze the concepts of creation of diversity, sorting diversity and splitting diversity into isolated phyletic lines by showing that these forces are founded on non-equilibrium thermodynamics and logical relations between the elements on which evolution operates. Legendre (1971) gives two diversity categories: a) chromosomal differentiation (speciation), and b) genic differentiation (intraspecific variation).



Within each form of such differentiation there is a hierarchical order that interconnects the two categories; e.g. the accumulation of genic differentiation could result in chromosomal differentiation. The correct assessment and understanding of the subject framework of both genetic and biological diversity are important for all forms of conservation science.

The genetic diversity within the world's plant genetic resources is an obvious necessity for the growth and improvement of crops in diverse environments. The results of stresses imposed by "pests" and environment on species of crop varieties that range from reduced productivity to extinction could be overcome using such resources of genetic variation (in evolutionary perspectives).

Mutation, selection, migration and drift are often considered as important causes in evolutionary changes of genetic variation. All of them could be treated as forces responsible for creation and sorting of diversity. Gene erosion is among the inevitable evolutionary changes in both wild and domesticated forms and is mainly caused by an interplay of these four evolutionary forces. The importance attached to these factors and their interplay for explaining the level of gene erosion and genetic structure of a population varies, depending on organisms studied, historical epochs of evolutionary synthesis and the various evolutionary schools of thinking. The gene conservation of wild species and primitive domesticates that carry the genes for characters desperately needed now and in the future, is a prime necessity to the whole

mankind. Evolutionary approaches in gene conservation should thus be faced regardless of their complexities and immediate economic return.

In situ gene conservation and conservation in gene banks are usually forwarded as two ways of overcoming the problems of gene erosion. In situ gene conservation is essential since it generates the establishment of new variation that provides different patterns of gene survival. Such variation and patterns of gene survival affect the potential for future evolution and adaptability under changing environment.

Any species is a product of a habitat, and the best way to preserve it is to preserve the habitat. Thereby the possibility of man's survival is enhanced through the understanding of rules and practices for conserving and managing these habitats. By gene conservation man attempts to direct evolution, no less by being aware of the impact of such events on his own evolution. Therefore, in situ gene conservation practices based on a single species do not seem to be a reasonable approach.

Pests possess a great genetic potential, i.e. great variability for damaging crops, and they are encouraged to produce certain mutants or genetic recombinations under environmental pressures or genetic uniformity (U.S. Dept. of Agric. Nov. 1973). In the past it was a common practice to breed crop plants for resistance against diseases without considering the genetic potential of the parasite. But at present the genetics of resistance is studied to-

gether with that of the parasite in order to explore more fully the genetic basis of the interactions in which host and parasite are involved. This should be taken up in relation with the problems of genotype-environment and population-environment interactions.

For genetic conservation work, measures of genetic variability and its pattern of distribution are essential. Such measurements should not only be within and across the species but also within and across phyletic lines. This will result in several ways of gene conservation and utilization of both intraspecific and interspecific variations of the various orders of gene pools.

The genetic diversity in the form of locally adapted land races and related wild species within the gene centers is a world resource of inestimable value and must be consciously conserved and utilized. This should take into account immediate fitness, adaptation within a phyletic line and speciation. The subject of conservation being just an applied science of evolution, it demands a proper understanding of adaptation by natural selection. This automatically is a challenge to measure selection and fitness in in situ populations and ecosystems which could be partially used to find important factors and indicators of stability parameters.

In situ gene conservation can not overcome, however, the inevitable gene erosion that is due to various evolutionary processes. It should be complemented with modern gene bank activities whenever possible. However, differential survival in storage, selection during rejuvenation, loss

of genetic integrity, and freezing of evolutionary processes in the samples of the gene banks call for some complement to the modern strategies of gene bank activities. Such complementary conservation activities can only be effective if carried out in a reasonable and, yet, limited scale. With regard to the domesticated plants, it is quite necessary to consider, first, the gene diversity centers of the various crops, and then split these gene centers into various microcenters. The further splitting of the center of gene diversity could be done by studying the genetic variation and their differential distribution by using some markers like isozymes, flavonoids and morphological characters. The pattern of variation as measured by means of these markers over the various microcenters could give an insight into the evolutionary forces responsible for maintaining genetic variability. It also could show how different genetically are individuals of a given species from different local populations. Such investigations are useful for managing endangered species by re-introducing individuals with constellations of adaptive genes particular to a local environment. However, the maladaptive genes present in the individuals may be adaptive when the success is looked at on the population level. It is necessary, for example, to maintain genetically diverse stocks in order to deal with continually evolving strains of diseases. Such genetic differences between individuals could result in the utility of different resources, thus reducing competition. Such reduction of competition may result, not only in bigger size of population, but also in time interval stable number of coadapted individuals. This, ultimately, could be used as basis for

various effective plant breeding works with land races.

The present work addresses a very central question to evolutionary biologists by taking the issue of modern gene conservation of land race cereals in terms of their

1. nature of genetic variability in a population,
2. magnitude of genetic variability in a population,
3. methods of maintenance of genetic variability,
4. evolutionary significance of the present variability,
5. future trends in the maximal utilization of such genetic variability.

The main objectives of the study are to:

- A. Estimate the amount of genetic variation maintained within land race cereal populations and the pattern in which genetic variability occurs among such geographically separated populations.
- B. Determine the degree and distribution patterns of multilocus genetic organization and association of different characters.
- C. Identify populations with unique character, for further theoretical and practical studies in the light of the recommendations to be given for managing the gene pool of the crops studied, and to have more insights into the nature of genotypic interactions. Multivariate approaches for quantification of genetic diversity in chemical and morphological characters within and among populations collected from different ecological zones will be used with the ultimate aim to arrive at a model for breeding in polycultural agriculture.
- D. Localize the Ethiopian centers of genetic diversity in

the form of microcenters in order to reduce the vague original areas into more specific sites for in situ gene conservation activities.

2. Genetic engineering vs natural variability

The reduction of genetic diversity can be thought to be partly overcome by techniques like genetic engineering, tissue cultures and chemical or radiation-induced mutations, and by alien chromosome transfer through conventional breeding. Such processes could hopefully be used with reduced problems in the future. This practical usage, however, partly depends on the magnitude of genetic diversity available.

Cavalli-Sforza (1978) describes genetic engineering as some form of hybridization and considers that since restriction enzymes are very 'natural', their role in nature may be, at least in part, similar to the one which is asked of them in the test tube. It is possible that DNA recombination has played an evolutionary role, for instance in intracellular symbiosis; e.g. mitochondria were the result of gene splicing from an intracellular microorganism and a eukaryote, where part of the spliced DNA may have been extruded as a ring in the cytoplasm (Cavalli-Sforza 1978). For other intracellular symbioses there may have been exchange of parts of DNA between host and parasite. Thus, Cavalli-Sforza emphasizes that genetic engineering does not replace nature as an inno-

vator, but just uses ways and means which are not commonly found in nature. Nevertheless, one should not expect that genetic engineering will solve all our problems in short time. Hopefully, it can contribute more to relieve some of the pressures which affect us today.

Genetically superior plants derived from modern crop improvement programs obviously require a high level of crop management. Included in this management are the input of increasingly expensive nitrogen fertilizers as well as the extensive use of pesticides and herbicides, all of which can result in toxic residue accumulation in the environment. The high degree of inbreeding and the narrowing of the genetic base of widely cultivated crops have resulted in high degree of susceptibility of crops to major disease outbreaks. Here recombinant DNA technology could be supplementary. However, the functional expression of a novel gene in a genetically engineered plant has not been reported because of the limited understanding of the molecular bases of gene expression (Barton and Brill 1983). Attempts to establish genetic engineering as a practical facet of plant breeding are also complicated by the fact that genes for most important plant characteristics have not yet been identified (Barton and Brill 1983).

Genetic engineering manipulates an existing genetic variability. The genetic variability generated during proliferation of plant cells in culture and that induced by mutagenic treatment can be examined for desirable traits at the two levels of differentiation (Chaleff 1983).

However, there are difficulties. One limitation, pointed out by Chaleff (1983), is that mutant selection in vitro can only identify modifications of traits that are expressed at the cellular level and the developmental complexity of higher plants makes this restriction rather severe. Many traits of agronomic importance are the products of the organization of highly differentiated cells and, therefore, do not appear in culture. And, because of rather primitive understanding of their molecular and cellular bases, correlative functions of these traits that may be expressed at the cellular level have not yet been defined. Thus, although certain agronomic traits, such as tolerance for heavy metals, salt, herbicides, and extremes of soil pH, may provide accession by an in vitro approach, others, such as yield, lodging resistance and times of flowering and maturity, are, at least for the moment, beyond the reach of the somatic cell geneticist (Chaleff 1983). However, the effect of genetic engineering in widening the genetic base of a given species should not be underestimated. Thus, for instance, protoplast fusion offers the opportunity of creating hybrid plants between related but sexually incompatible species (Shepard et al. 1983) and the frequent existence of chromosome translocations in plants regenerated from mesophyllprotoplasts could accelerate genetic variation (Shepard et al. 1983).

Commercial potato cultivars can not be crossed with many related Solanum species without first passing through a bridging species such as Solanum megistacrolobum. Since many primitive Solanum species possess broad-spectrum

resistance to diseases (e.g., S. etuberosa for resistance to leaf roll virus), protoplast fusion might allow a rapid introduction of resistance genes into potato germplasm pools. The merits of genetic transfer in plants through interspecific fusion are presented by Shepard et al. (1983). In true interspecific crosses between most distant sexually compatible species considerable backcrossing is needed to eliminate unwanted portions of the alien genome. Hawkes (1983) gives the following four screening steps in search for adaptation and resistance characters:

1. Working and other collections, national breeding stocks, breeding lines, mutants, and current and obsolete national and exotic cultivars.
2. World collection of land races and primitive cultivars.
3. Related wild species and weedy types.
4. Intergeneric hybrids.

The above categories correspond well with what Harlan (1975) termed as primary gene pool (Gp-1, e.g. no. 1 and 2 above), secondary gene pool (Gp-2, e.g. no. 3 above) and tertiary gene pool (Gp-3, e.g. no. 4 above). Harlan (1975) discussed the differences in the gene pool categories between the various crops. The utility of Gp-2 and Gp-3 is usually not easily attainable because of difficulty to cross, sterility of the hybrids, differences in the ploidy level and wild genetic background (Hawkes 1983). Such difficulties could be overcome only in a very few cases (e.g., Müntzing 1966; Merker 1984; Dionne 1961, 1963), even in the advent of modern techniques.

Novel somatic hybrid plants are only the starting point

of a genetic introgression scheme. If the hybrid plants are sexually compatible with either parent, removal of non-desirable material can be straightforward. Where this is not true, protoplast regeneration from interspecific hybrids that undergo continuous segregation should provide novel genomic mixes (Shepard et al. 1983), thus adding more variation. Nevertheless, it is not difficult to imagine that the amount of the variants of genomic mixes in interspecific hybrids depends on the magnitude of parental gene variation. However, in interspecific protoplast fusion there is little control over what genetic information is retained and what is eliminated, and fusion lacks the potential precision of the recombinant DNA method (Shepard et al. 1983). Until the process of directed transformation with cloned genes reaches a higher level of sophistication, protoplast fusion offers a means of introducing genes from unconventional sources, but by maintaining their variation. In addition, we should not neglect the more conventional areas of plant breeding research, since they represent the major line of defence today on the food front (Borlaug 1983). Besides, the advent of genetic engineering gives an insight into the importance of in situ gene conservation. Genes could be transported between different species by viruses, e.g. the barley stripe mosaic virus (BSMV). The BSM-virus is a seed-born virus and the only seed-born virus known in the grass family. Although the spread of this virus from diseased to healthy plants is accomplished through leaf-to-leaf contact, its transfer through pollen has also been demonstrated (Sandfaer 1977). This makes the transport of genes very effective.

Such a case might have been of importance in gene variation of cultivated plants. This, together with environmental selection and evolutionary relationships, could explain Vavilov's law of homologous series and accumulation of genetic variants in gene centers. It makes, however, classification of gene pools ambiguous and a rare distant hybridization explainable.

3. Historical development of gene center concepts

Geographical centers of genetic diversity have had historical roles in crop evolution and as sources of germplasm for breeding and genetic conservation. The concept of genetic variation vs center of origin has received criticism since de Candolle. The centers of genetic diversity and origin as defined by Vavilov are still being used. Since various criticisms have been made on these centers, a presentation of these critics and further subdivision of each center of genetic diversity is needed to define concepts of microcenters for gene conservation.

Alphonse de Candolle (1844 (1959)) made a special study of cultivated plants and their history. de Candolle's greatest contribution was to focus attention for the first time on cultivated plants, and he laid down a series of percepts or suggestions for their study, pointing out pitfalls in the various methods. Many of his conclusions are untenable at present day, chiefly because of advances in genetics and cytology, as well as in com-

parative phytochemistry and in archaeology. Furthermore, he did not examine the botanical details of the crops sufficiently. However, his latter work was a masterly review of the historical, linguistic and archaeological data available at that time. de Candolle noticed that in many regions of the world in primitive times valuable crop plants were largely absent; this was especially so in Australia, South Africa and North America, because of the lack of suitable plants and/or the people of those regions not being sufficiently advanced to have arrived at agricultural stage. de Candolle thought that agriculture did not originate in one place and spread later to other parts of the world, taking other plants into cultivation as it arrived.

Nikolai Ivanovich Vavilov is mostly remembered for his theory on the origin of cultivated plants. His search for new varieties forced him to make a series of worldwide expeditions. The results of these collecting expeditions indicated that there existed a concentration of genetic variation for each crop in one or a few regions of the world. These regions were to be found in the tropics and subtropics and mostly in mountainous districts where there is a great contrast of ecological conditions. Other regions, however, notably Australia, North America and Northern Europe, were characterized by scarcity of primitive varieties. The areas of varietal diversity he termed "centre of diversity". Initially, Vavilov recognized eight centers, and he suggested that these areas represented the "center of origin" of the crops concerned. His suggestion was based on the as-

sumption that the longer a biological entity occupies a given area, the bigger the number of variables it would produce. This, however, seems an oversimplification since the build-up of variation in the majority of biological entities proceeds at different paces in different places.

The fact that genetic variation is accumulated in some regions, even if the crop is not originated here, brought the need to differentiate center of origin from center of genetic diversity. For the former, primary centers, and for the latter, secondary or accumulation centers are sometimes used.

According to Sewall Wright's model (1943) an ideal situation for rapid divergence would be given by a collection of small populations partially isolated from one another, and each occupying its specific niche. Mountain areas with their spatially semi-isolated farming communities and cultivation patches on one hand, and with their wide amplitude of climate and soils on the other hand conform very much with the requirement set up by Sewall Wright's model for rapid evolution. Here apparently lies, at least in part, the explanation for Vavilov's "secondary" centers of origin. Stochastic fluctuations in allele and genotype frequencies would be better preserved by the subdivided population than if the population were panmictic, thereby building up variation. But, this is only part of the story because one usually finds, in these areas, an enormous diversity in a single cultivated field. There is much more to a center of diversity than a variety of ecological situations and population subdivisions (e.g. gene transporta-

tions by viruses and gene flow with linkage and epistasis).

Harlan (1975, p. 149) gives the following points as some observable factors that cause variation to accumulate in secondary centers:

1. A long history of continuous cultivation.
2. Ecological diversity, many habitats accommodate many races.
3. Human diversity, different tribes are attracted to different races of crops.
4. Introgression with wild or weedy relatives.

He summarized the reasons for secondary centers as human, environmental, and the internal biological dynamics of hybridization, segregation and selection.

It has been a pre-occupation of many workers to redefine the exact meaning of "centers". For example, Zhukovsky (1969) thought the situation could be best represented by megacenters. But these covered much of the earth's surface and hence were not really centers at all, and were not helpful. To draw a line across a whole continent and call it a center is to misinterpret the concept of center. Darlington (1969) agreed with Vavilov's ideas, and added several more centers to the original, making a total of twelve.

More recently, Vavilov's centers have been criticized for being too large and too vague. Hui-Lin Li (1970) considers Vavilov's centers to be extensive geographical areas rather than centers as such. Thus, he criticises the boundaries of the centers but not the philosophy behind them.

He noted in particular that the Chinese center is particularly large, embodying much latitude, and conjectures that these centers seem to have been decided on political rather than botanical grounds (though of course political barriers can act as barriers to the spread of cultivation). He also thinks that Vavilov was unfamiliar with this particular region since he listed plants belonging to that center which can not really be considered to be cultivated, such as Aconitum wilsoni. Hui-Lin Li takes the view of modifying the concept of centers into "regions of collective origin" of the major crops, which should be contained within the natural area of distribution of the wild progenitor.

Harlan (1971) also considers Vavilov's centers to be too vague a description. He states that "agriculture may originate in discrete centres or evolve over vast areas without definable centres". He questions the traditional concept of centers since it is not unusual for the center of diversity to occur far from the center of origin. Harlan (1975, p. 150) gives the following categories to describe the concept of center as evolutionary patterns that differ not only in both space and time but also among crops that have different evolutionary patterns:

1. Endemic: crops that originated in a limited area and did not spread appreciably, e.g. Ensete ventricosa in Ethiopia.
2. Semi-endemic: crops that originated in a definable center and with limited dispersal, e.g. Eragrostis tef and Guizotia abyssinica.
3. Monocentric: crops with a definable center of origin

and wide dispersal with secondary centers of diversity, e.g. Coffea arabica.

4. Oligocentric: crops with a definable center of origin, wide dispersal, and one or more secondary centers of diversity, e.g. Brassica spp.
5. Non-centric: crops whose pattern of variation suggest domestication over a wide area, e.g. Sorghum.

Vavilov himself recognized the problem when he established the concept of secondary centers, but it is still useful to classify the variation within a cultigen and plot the geographical distribution of variation. Nevertheless, patterns of variation must be analyzed since they provide significant information for the basic understanding of gene centers and conservation.

Harlan (1971) proposes the independent rise of agriculture in three separate regions, each consisting of an interaction of a center and a non-center. One system includes a definable Near East center and a non-center in Africa; another system includes a North Chinese center and a non-center in South-East Asia and the South Pacific; the third system includes a Meso-American center and a South American non-center. But this definition seems to be changing because of recent archaeological findings of a remnant of agriculture in the Nile basin, Southern Egypt, that traces back to 20.000 years.

Harlan (1951) mapped microcenters in Turkey and Asia minor and concluded that the structure of Vavilov's centers is subject to study, since they contain regions of

varietal paucity as well as regions of varietal wealth. He suggested that the problem should be considered on a more local scale by dissecting the center into various anatomical levels of genetic variation. Darlington (1969) agrees that regions of genetic diversity are places of development, not of origin. Not only can a particular crop have several areas where it exhibits great diversity, but also evolution in each crop may proceed at vastly different rates depending on where the crop is grown. Thus, the region where it shows most diversity is not necessarily the region of its original domestication.

Leppik (1968) supports Vavilov in that centers of diversity can be substantiated by relating to the incidence of fungal disease.

Many workers as shown above have indicated that the concept of the existence of centers of genetic diversity is an established fact, though the exact interpretation is a matter of controversy. However, the general notion underlying them remains basically unchanged, particularly if it is possible to localize these centers in the form of microcenters and reduce the vague original areas into more specific sites. Such an approach facilitates the future utility of evolutionarily derived variation which could only be accessible in in situ gene conservation.

4. Plant breeding selection problems in land races

The whole success of a breeding programme depends on the efficiency of the selection technique. Ease of selection depends on the kind of gene (e.g. monogenic vs polygenic), the genetic background of the gene, and the complexity of the population selection being carried out.

No selection procedures could overcome the problem of interacting genotypes in base populations. This remains as a plant breeding dilemma since Sakai (1955). However, there is a revival of interest in this topic, and some approaches have been developed. Williams (1962) observed that the analysis of mixtures in competition experiments could be similar to those of diallele cross designs. Harper (1965) extended this idea further by producing a type of diallel graph for arrays of mixtures, which seemed capable of identifying intervarietal interaction in such a way that non-allelic genetic interactions are revealed when hybrids are used. Further work was due to Allard et al. (1966), who gave an account of the deviation of "selective values" in mixtures of pure lines. Recently, Griffing (1976a, b) provided an alternative theoretical solution by considering three strategies involving individual, group and index selection. There might be a tendency to break back into agriculture with some forms of mixtures (Walker 1969). Such breaks, however, should not be based on highly specific fragments. It should consider some relevant information in the magnitude of genetic variation and structure of land races both in time and space.

Land races are mixed genotypes which have built-in safeguards against epidemics in that no race of pathogen can attack all the genotypes, although no genotype is resistant to all races of pathogen (Harlan 1975). This provides some built-in resistance against several stresses. Allard (1970) pointed out that in natural populations and old land races a high degree of genetic variability and polymorphism is found in both discontinuously and continuously varying traits. Although these land races are genetically complex, it is of interest to consider how to construct a modern version with similar broad adaptability but with higher productivity.

Genetic variation within a land race is considerable, some of it being far from random. Here, the various genotypes of the composite populations have survived for a long period of time and such genotypes may reasonably be assumed to be not only adapted to their environment, both natural and man-made, but also adapted to each other. Such interacting genotypes display differences in competition and, perhaps, coadaptation which could be utilized when the underlying mechanism is better understood.

When we use Jink's (1978) terminology on the classification of genotypes, land race composite populations at first sight seem to be composed of all or any combinations of the following:

- a. Unresponsive genotypes
- b. A tolerant or well buffered genotype
- c. A stable or reliable genotype
- d. A highly adaptive or flexible genotype.

However, any combinations of the above to be taken as a characteristic of the defined genotypes in the composite populations may not hold true, if those genotypes are taken and tested individually. There is thus a need to consider the nature of the complexity of the interacting genotypes in such composite populations before embarking on any schemes of selection. It is, e.g., essential to know whether favourable environmental conditions will enhance the expression of a desired character in one group of genotypes with respect to more gain from selection than will unfavourable conditions in another one or group of genotypes.

The differential responses of various groups within land races is an indication for the existence of complex classes or groups. Some of the classes could be treated as a closed group, which means that genotypic interactions are confined to members within groups and that the position in the group does not influence the magnitude of interaction. Some classes may be treated as an open group model that permits interaction between plants inside and outside a given group. The results of selection on the open group model are more complicated because more than one class of associate effects must be considered (Griffing 1976a, b).

In land race populations some genotypes are dormant. Dormancy will lead some seeds to germinate a little earlier than the surrounding seeds and probably give the resultant plants the advantage of an earlier start and development at the expense of the neighbouring plants (Sandfaer

1970). This results in comparatively higher performance of that particular genotype which may not be true if the genotypes are considered by taking away the masking effect of dormancy. It is possible that differences in performance can be reversed so that the best performing genotypes in non-competitive conditions become the less fit when competition sets in. Ignoring such competitive effects will also result in a higher estimate of genetic variance leading to misleadingly higher heritability estimates, which are used to plan the whole breeding programme. Phenotypically extreme individuals are less likely to survive and reproduce than typical individuals (Cavalli-Sforza and Bodmer 1971; Fox 1975; Franklin 1980) and that fitness is therefore inversely related to an individual's deviation from the average character state for quantitative traits.

In the original site the composite land races are in equilibrium with various races of pathogens. It is important to know the composition of the host and races of pathogen for effective control of the pathogen in its original site. To recommend varieties that are resistant prior to race composition study, and replace the land races, may result in selection pressure and other intricate factors to favour the fittest evolving parasite, which may even wipe out the already existing resistant types in the gene bank. This calls for annual observations of races to display differential biotype occurrences. Here, there are two points to be noted. First, the percentage occurrence of each component in the composition of the races of pathogen varies with season and

locality. This implies that unless the time and place for a record is designed to cover a sufficient range of distribution of both the host and the parasite, the selection performed will be incomplete. Secondly, sites of high genetic variation for the host and the pathogen coincide. The two participants have developed a complementary genetic system (Flor 1955) that governs their behaviour. For successful resistance-breeding, knowledge of both genetic systems (e.g. virulence vs resistance) is important for selecting the "stable" genotype(s). Such detailed studies might even lead to a stage where it is practical to select and breed given races of the pathogen that are aggressive competitors to other pathogens. The presence of intergenotypic competition in both host and parasite populations implies, as Mather denoted it, that each competing genotype is an effective part of the other genotypes' environment (Mather 1961).

The frequency of the various genotypes and their co-occurrence vary across the ecological gradient (II). However, in each ecological gradient only some are highly productive (Zemede 1980). Whether this differential productivity is due to competition or genotype-environmental interaction or the combination of both is not clear. Mather (1961) considers competitive ability as a result of many phenotypic features of the individual, which implies that different characters are added up into one character, which is the competitive ability, and that the share of input of each individual character into competitive ability varies with the environmental factors. Nevertheless, the result of selecting highly productive

genotypes out of a land race does not correspond with the expectation. It is possible that differences in yield can be reversed so that the best yielder of genotypes in non-competitive conditions becomes the poorer yielder. This might be due to:

1. Intraspecific competition.
2. Coadaptation shared with other low yielding types in the environment.
3. The specificity of the environmental factors in that ecological gradient.

Bennett (1970) reported that local selections of Triticum aestivum L. which were widely distributed in dry farming areas of the Northern Badakhashan region of Afghanistan, were equal in yield to the best introduced high yielding Mexican wheats and far surpassed them in quality and germinability of the grain. Dorofeev and Jakubziner (1973) indicated that old local durum wheats that evolved by human selection through different regions of the USSR possessed valuable biological and economic characters with striking adaptability to local growing conditions.

High yielding genotypes may not always be stable when they are grown alone. This implies a negative relationship between the two dimensions of competitive gene action, "direct" and "associate". Positive individual selection may result in a negative change in the mean. If such selection is continued, it could result in fixation of the least desirable genotype. This suggests that individual selection from a randomly planted composite population of land races is not advisable. In such a land

race genotype community the inferior genotype is important to favour the fitness of its superiors and, thereby, the population as a whole. Gustafsson (1953), in his study on monohybrid offspring of ert-29, AaBB in barley, as well as the Golden, Maja, and Bonus barley mixture showed that a population lacking inferior genotypes may be less fit in total production than a corresponding population hiding genes that cause less individual fitness. Parallel observations in advanced forms were made by Wiebe et al. (1963), showing that an aggressive competitor has an advantage in mixed stands but yields poorly in pure stand. However, the high yielding forms in land race populations are the result of long evolutionary processes that may be dependable. But the utility of such forms could only be settled after a study of the level of genetic variation, and the patterns of its distribution. Such an approach should consider the structural and functional complexity of the populations and how these change with environmental factors. With the information abstracted from such a study, and supplemented by implications from theoretical considerations for accommodating genotypic interaction in artificial selection theory (individual, group and index selection), procedures operating on random and non-random group structures as presented by Griffing (1976a, b) might be applicable.

5. Genetic variation and evolutionary gene conservation of land races

Plant domestication is an ongoing evolutionary process throughout the history of mankind. During this process of domestication in different environments, with varying selection pressure including farming techniques, rapid evolution with continual change has taken place. Such a continual genetic change of domesticates is complementary with genetic changes of their progenitors and parasites. This complex genetic build-up should be the basis for plant breeding procedures and also suggest the value of in situ gene conservation. Although the importance of biology of land races is discussed and their importance in plant breeding and gene conservation appreciated (Harlan 1975; Frankel and Bennett 1970; Frankel and Hawkes 1975; Frankel and Soulé 1981), the way to continue conservation as an evolutionary process in terms of its long-term effect is not yet clarified.

Hawkes' (1983, p. 113) statement that we are not certain what we shall need in the future is a statement of caution, calling for what should be taken in line with priorities and strategies in gene conservation activities. It suggests additional recommendations and challenges to the successfully proceeding, well organized world system (IBPGR) that coordinates conservation activities. Bennett's view (1968, p. 21) implies that the primitive cultivars and the variable conditions in which they are grown can be used to recover the genetic variability which has been accumulated by the evolutionary explosion

of the past. Her paragraph reads as follows:

"Not only is the modern cultivar genetically uniform. The cultural conditions of modern agriculture are becoming increasingly uniform. The exceptional conditions for successful hybridization, recombination, segregation, introgression, and mutation created by primitive cultivation are now giving way to conditions which prevent such genetic events. Genetic erosion is thus accentuated."

The paragraph cited above should consider the previous implication. Otherwise, Bennett's suggestion (1965) becomes unclarified, when she points out the urgent need of increased knowledge of ecotypic diversity of species in order to overcome the fragmentary knowledge of their genetic potentialities.

Frankel and Soulé (1981) discussed the consequences of bottleneck effect on the reduction of genetic variability, particularly when the bottleneck event is perennial. They used

$$E_{(n)} = m - \sum (1 - P_j)^{2N}$$

as a measure to estimate the number of alleles (n) remaining after a bottleneck, where

$E_{(n)}$ = number of alleles remaining after a bottleneck,
 m = the number of alleles prior to bottleneck,
 P = the frequency of the j^{th} allele, and
 N = the effective number of individuals at the bottleneck.

In cereals, throughout the center of genetic diversity,

drought has resulted in a bottleneck, in some cases perennially. Frankel and Soulé (1981) and Nei et al. (1975) pointed out that the rate of population growth plays a role in reducing the post-bottleneck loss of variation. Although Frankel and Soulé (1981) do not recommend in situ gene conservation of land race cereals, their various points of view given throughout their book do, indeed, suggest the importance of in situ gene conservation, not only for wild, but also for cultivated types.

The modern detection of variation for gene conservation works of any taxonomically defined local forms has revealed that variation differences between populations are very common and exist even among adjacent populations (I). Populations could differ, either in the frequencies of some marker alleles, or in the type of alleles present (I). The two alternatives could be stabilized or change in the members of populations considered, depending on the intensity of selection and gene flow. The distribution of the population in the form of "island model" (exchange of pollen or genes with all others) and "stepping-stone model" (only neighbouring colonies like clustered populations) also bring about changes in the differences in frequencies and types of alleles among populations, particularly in outbreeders. Allelic fixation differing among populations (I) could result by isolation in space, caused by topography, distance and limited outcrossing. However, this event is counteracted by mutation, heterozygote advantage and disruptive selection, all confounded with human activity in cultivation.

Although the magnitude of gene erosion could be in reduction, endangered or extinction phase, Denniston (1978) asserts that most crop plants are in endangered phase (i.e. incipient extinctions or hopeful bottlenecks). For some genes they are also in extinction phase. According to Wright (1931) increase in population size adds new mutation and thus the endangered phase of the land races studied proceeds to a recovery phase which is only possible in in situ gene conservation activities. In situ gene conservation could also be used to provide information how a population of plants becomes adapted to different regions. This in turn could be used to utilize the new derivatives of existing variability in the improvement of crops. It could also be used to generate variability by consciously capitalizing on the causes and mechanisms that resulted in the present variability (II).

Crow and Kimura (1970, p. 256-258) have given several factors for the maintenance of gene frequency equilibria. From the presently studied barley populations, the following factors (perhaps singly or in several orders of combinations) are considered as forces maintaining polymorphism:

1. Balance between mutation and selection (VI).
2. Heterogeneous environment (I).
3. Neutral polymorphism (IV).
4. Balance between selection and migration (II).
5. Frequency dependent selection (II).
6. Transient polymorphism.

Natural selection is an evolutionarily logically related compound factor in time and space. Together with genetic drift it operates on evolutionarily related genetic backgrounds distributed over environmental gradients that vary in time and space. That means that variation of a given trait can not be directly explained by genes that account for its inheritance, nor can it be correlated at ease with any environmental factor in space at any time. But from the five enzymatic loci studied (I) the observed regionally defined multilocus organization (IV) and genotypic distribution (II) could be due to these marker loci monitoring the selection flux distributed throughout the genome. This could be further substantiated by VI where enzymatic studies showed disturbed trends of correlations with morphological and flavonoid variables.

The observed multilocus genetic organization (IV) could also result from the smaller than expected cross-over values between the three linked esterase loci, which could let the alleles act as a single unit to form a supergene. According to Ford (1975) such a supergene is a device that holds co-adapted groups of genes together. Such supergenes could vary in the level of their intensity, by cryptic variation or environmental segregation. This could, perhaps, form a background for what Ford (1975, p. 157) terms as the switch gene in which selection operating on genetic variability can alter the effect of a gene so as to make it either dominant or recessive. Ford (1975, p. 158-159) showed how the dominance in the butterfly Triphaena comes breaks down because of identical switch genes with different modifiers that

adjust dominance. Thus, polymorphisms may differ among one another, depending on the genetic system.

Modern studies bring isozymes into key position not only in gene conservation works but also in the evaluation of the utility of the genetic materials. Isozymes have been used by many workers (e.g. Kahler and Allard 1981; Almgård and Landegren 1974; Nielsen and Frydenberg 1972) to assess the genetic status of collected germplasm. Such information could be used to partition the subsamples of the population in the gene bank. This approach should aid in a more efficient use of germplasm in evaluation and breeding (Brown and Clegg 1983). It is also important to find regions of rich genetic diversity for re-sampling (Brown 1978). Isozyme variation could also complement DNA variation. Thus, Brown and Clegg (1983) reported that the barley chloroplast DNA from four cultivars, five land races, and ten Hordeum spontaneum collections after digestion by various restriction enzymes showed variation only among the land races and Hordeum spontaneum. These patterns of cp-DNA variation corresponded well with the observations of isozyme variation (Brown and Munday 1982).

Isozyme variations have been used as markers to detect and map genes controlling quantitative traits. The relationships between characters determined by major and minor genes on the one hand, and morphological variation and enzyme variation on the other, are not always clear. This is partly due to the genetic backgrounds and the gene expression of the two classes of genes being confounded

with various ecological variables (VI). However, there are enough evidences to use isozymes as reliable markers for evaluating the various models of quantitative genetics. Thus, there are some data from plant and animal populations in literature suggesting that individual genes with major effects account for much of the genetic variation in some quantitative characters (see, e.g., VI). This could give an immediate insight into the nature of quantitative inheritance at the genic level, in order to manipulate the genes and change quantitative traits in desired directions through breeding programmes. However, such a relationship seems to be more operational if the present electrophoretic procedures could quantify the expression of the various alleles rather than leading to the usual qualitative estimate. Such an approach could also show how different strategies of adaptation among very different kinds of organisms could be looked at very specifically in terms of protein and morphological variation.

The observed cryptic variations (environmental segregation) (V) are useful to the organism only if they create a flexible system to adjust the organism to an ever changing environment. The magnitude of genetic variation in the population may be attributable to such flexibility in such a way that a locally variable population is composed of individuals with flexible biological systems. However, how such flexibility could induce variability under a given set of environments is difficult to account for from the present study.

In the land races studied here a great deal of diversity

is present despite the high degree of inbreeding, possible founder effect at the time of initial introduction, and genetic drift that is due to reduction in population size. Such high magnitude of variation can aid in fixation of saltations (Willis 1981, p. 12) as suggested also by Bekele (III). Under such high genetic variation, drift and recombination coupled with artificial selection could result in adaptive combinations of genes in the form of Wright's (1970) "shifting balance theory" where he pointed out that recombination between epistatically interacting complexes of genes will often produce very unexpected results by chance. Thus, the observed distribution of variation in the presently studied domesticates is the result of evolutionary processes that are due to both chance and selection (IV). However, how much chance and how much selection is acting on each population is difficult to quantify. Besides, the meaning of chance event is an unclear issue.

The meaning of chance or randomness is a problem that has preoccupied philosophers and mathematicians for centuries. It is not always clear what is meant by the usage of chance or randomness. Chance does not mean without a cause. Even in the simplest common example of flipping a coin, it is the force that is applied, the way the coin is held and the gravitational pull which all determine how the coin falls. It is only because we are ignorant of quantifying these physical forces that we attribute the outcome to chance or randomness. Thus, the process of mutation may be taken as an important way through which chance influences evolution. But mutation, like any other event of its kind, has its own knowable and

presently unknowable physical causes, although it is problematic whether it is possible at all to have enough knowledge of anything for perfect prediction of all the causes (VI). The above philosophical dilemma on what chance means, does not, however, result in the concept that evolution of organisms is multidirectional, since any preexisting organism is restricted not only by multievolutionarily required genetic resources but also by existing structures and functions.

The regional specificities of multilocus structure (IV) is inconsistent with genetic drift of neutral alleles, migration and mutation. It suggests that selection is determining the gene frequency patterns and gene association in these regions. It also shows that selection favouring allelic linkage blocks maintains allozyme polymorphism. The ubiquitous genotypes (II) over populations and regions could be mainly the results of balancing selection rather than only migration.

From the analysis of altitude regression on gene diversity (I) it was found that clinal variation is absent or rare. This indicates that mosaic patterns are characteristics of spatial organization of polymorphism in these land races. The lack of clinal variation is perhaps due to population number, migration, founding event, interaction and fluctuation of local selection factors disturbing the possible linear relation between altitude and gene diversity measures.

The analysis of hierarchical gene diversity of the land

race populations of barley (I) were computed according to Nei (1973, 1975). In each population the gene diversity per locus is denoted as

$$h = 1 - \sum x_i^2,$$

where x_i is the frequency of the i^{th} allele. The mean gene diversity of each population over the loci considered is

$$h_S = \frac{h}{n},$$

where n = number of loci studied.

The total gene diversity could also be computed by considering the unweighted average allele frequency over all the population. This is decomposed into

$$H_T = H_S + D_{ST},$$

where D_{ST} is the gene diversity due to differences between populations. D_{ST} is further decomposed depending on the levels of hierarchy used. In the present case

$$H_T = H_S + D_{Spa} + D_{SAR} + D_{SR}$$

$$H_T = H_L + DL_A + DL_R + DR$$

The contributions of each hierarchy are expressed in terms of H_L/H_T , D_{LA}/H_T , D_{LR}/H_T , D_R/H_T .

Such hierarchical approaches are the key factors to quantitatively define a center of genetic diversity into micro-centers. At present a single species approach is followed. But such comparisons should be made also among different species and among different pathogens at the gene and

allelic levels to see the coevolutionary gene communities of crops and their pathogens (I). For evolutionarily oriented gene management at the microcenter level and for practical plant breeding works such an approach is appropriate. Thus, Bekele (I) discusses the importance of identifying populations rich in alleles in each microcenter, because of their usefulness for conserving germplasm resources. The choice of genetically contrasting populations may facilitate selection for high levels of recombination and thus the ability to generate new and more adapted genotypes in a study of the physiological and ecological consequences of different degrees of genetic variability. From the investigated samples of barley populations the supply of alleles uncovered for the isozymes studied varied among regions, areas and localities. This, together with varying contributions of the various levels in the hierarchy, indicated differences in conservation strategies to be developed for each level of hierarchy, as it was discussed (I). The observations also implied a differential requirement of genetic base and heterogeneity for breeding genetic types for different levels of the hierarchy. Land race cereals, though low-performing, are usually stable in their performance. This, among other factors, is taken to be due to their limited distribution and the balance reached in their genetic composition (II).

The allozyme genotypes detected are not homogeneously distributed except for two widely distributed genotypes. Some genotypes are location specific because of gametes and alleles that interact well with each other, in the

form of multilocus combinations against a multitude of genetic backgrounds (II).

The null alleles at the Est-B locus are presumed to be facultatively lethal, and maintained by the genetic background. Their origin is probably a case of Sturtevant's hybrid dysgenesis, which partly explains Vavilov's emancipation of recessives (VI). The fixation of null alleles at the Est-B locus also gave the plausible implication that the three linked esterase loci are due to some form of gene duplication (VI).

The effect of null alleles and the correlation between variation in traits controlled by major and minor genes are assumed to reflect different strategies of adaptation among very different kinds of organisms. This perhaps suggests the possibility that protein variation and morphological variation have different significance in different organisms with widely different organization and ecological situation (VI).

The allozyme data revealed a non-randomness in multilocus genetic organization which is of importance as a marker for genetic management of a given gene pool. This should take into account how to utilize the results of evolutionary processes, which in turn demands the appropriate resolution of evolutionary controversies and their impacts on the methodologies of gene conservation (IV). Bekele (IV) recommended a follow-up of the evolution of such coadapted multilocus structures in centers of gen-

etic diversity for several hosts and parasites. This could be used in order to understand the evolutionary dynamics of the adapted forms and the management of a given gene pool. The observed multilocus association also suggests that collection and rejuvenation of germ-plasm should be done in such a manner as to preserve their associations. It also bears an impact on plant breeding methods, since standard selection schemes assuming additivity can not predict the effect of the behaviour of interacting multilocus systems.

The regional multilocus genetic organizations observed vary in their intensity across the regions (IV). The variation in intensity of multilocus genetic organization is equivalent to variation in linkage intensity, and, thus, can be explained by factors that have a role in the modification of linkage intensity. This could be brought about through structural changes such as an inversion or translocation that brings two genes (earlier far apart or on different chromosomes) close together. It could also be brought about by modifier genes controlling the rate of recombination between linked loci.

Although an individual's fitness (viability) may not depend on its genotype at the modifying locus, the frequency of an allele at the modifying locus may change because of its statistical association with alleles from the primary locus. But this does not tighten all the loci, both linked and unlinked, to a coadapted unit. What is required is a selection pressure at the linked loci that maintains a stable polymorphism at both loci and also

produces a non-zero disequilibrium coefficient, $\hat{D}$, (Nei 1967; Karlin and McGregor 1974). If this condition is met, then any new modifier allele that tightens the linkage will spread.

The regionally different multilocus structures and genotypic compositions observed (II, IV), if taken with their respective possibly defined multilocus structure of parasites, will be of high interest in any selection schemes in each micro- and megacenter of the regions studied.

Sites of in situ gene conservation are of advantage also for the endemic balance which prevents pathogen outbreak. Thus, such sites are additional checks of endemic balance resulting in the stability of some of the introduced modern cultivars.

Polygenically inherited variability of a population is not best estimated in the parental environment. This may be partly due to intricate expressions of genes in different environments (Turesson 1922; Clausen and Hiesey 1958). Such differential expressions of intricate effects of genes result in heritability changes. Differing heritability in different environments of a given character or a given series of populations can be used to identify the level of ecotypic differentiation.

Both tetraploid and hexaploid wheats (V) were taken to have a particular adaptive strategy to meet the challenge of environment through the effect of correlated characters. Combinations of characters were also found to show

differences among regions and between ploidy levels. Such observations could be looked at in several alternative ways. The correlation of characters could be quite neutral. It may very well be directly or indirectly linked to other characters, of adaptive significance for the two ploidy levels of wheat. Natural selection is usually taken to act on many characters favouring the optimum types with harmonious combinations of variants of characters in each peak of adaptation. But the effect of character state combinations could change, depending on the type of genotypes considered, the nature of genotypic composition, and interaction (II).

The observed character combinations in all these cereals studied could be unambiguously ascribed to different types of selection only if they could be followed for many generations with at least some genetically defined marker genotypes. Even under such circumstances, several questions could arise. The major factor in such processes of correlated morphological change and cryptic variation (V, VI) is their relationship to adaptive evolution. However, such usually a priori conclusions need strong supporting evidences from the in situ gene conservation activities.

In some areas, there is population fragmentation by human activity. Thus, the same population could be split into various units to be grown in separate fields even by the same farmer. This ultimately results in differentiation among subpopulations. Harlan (1975) considers such differentiation among subpopulations to partly break down because of hybridization. Accordingly, the differentiation-

hybridization cycle adds diversity in such a way that distribution of variation becomes complex. The degree of differentiation depends on the amount of buffering in the genetic system (Harlan 1975), i.e. the amount of redundancy of genetic information. However, the variations observed in barley (VI), and tetraploid and hexaploid wheat (V) do not give a clear picture when the coefficients of variation of quantitative characters are compared for their ploidy levels. This could partly be due to generally lower outcrossing. Nevertheless, high crossing in some regions could result in a high major release of potential variability, first in diploids (since here it is weakly buffered), being followed by tetraploids, and finally by hexaploids. In the diploids the variability should be largely oligocentric, in which relatively few genes have conspicuous effects and, truly, wide hybridizations should be disastrous, if at all possible (Harlan 1975). The comparable high variability of barley (VI) should thus be considered very cautiously and bring into focus partly why the parallelism of variation of different crops in terms of Vavilov's law of homologous series is not always clear. Clarity could perhaps be gained if mating system and ploidy levels are also considered in such comparisons.

6. Problems of sample size in gene conservation

The sample size techniques and requirements for gene conservation works are very variable (Marshall and Brown 1975;

Bennett 1970; Jain 1975; Qualset 1975; Adary 1978). Thus, the number of individuals to be collected from each population as based on diverse experiences and purposes is also variable. This can be seen from the following recommendations.

- Bennett recommended 200 - 500 spikes per population.
- Marshal and Brown recommended 50 - 100 spikes per population.
- Qualset recommended 500 spikes per population.
- Adary recommended 50 spikes per population.

Among the important points excluded in these recommendations are the history of the populations, i.e. demographic changes at time intervals, and the area occupied by each population and the kind of statistical distribution of individuals or combinations of genes. Since sampling of individuals in the field is without replacement (unlike the binomial distribution) the probability of picking each of the different types is not constant at each stage of picking the spikes and depends on the size of the population. At each stage the probability of picking the already picked group decreases while the unpicked ones increase. Such changes of probability at each stage because of absence of replacement involve hypergeometric distribution. A more general hypergeometric problem may be stated as follows (Steel and Torrie 1960, p. 388):

"Given N elements consisting of N_1 with property A and $N - N_1$ with property not A , the probability that a sample of n elements, drawn without replacement, will consist of n_1 with property A and $n - n_1$ with property not A could be given by:

$$p(n_1) = \frac{\binom{N_1}{n_1} \binom{N - N_1}{n - n_1}}{\binom{N}{n}}$$

Cultivated forms are under humanly treated "controllable" environmental factors even in the primitive agricultural system where they exhibit semi-wild character. The tilling of the field, the site choice, the aspect choice, the season choice are conscious attempts of homogenizing the environment of the individuals of the population, unlike the conditions present in the wild. Because of the differences of such trials in homogeneity and uncontrollable factors, the population evolves structural variation to stand the environmental challenge. Generally, cultivation reduces the infinite diversity of natural environment to simple dimensions and changes the operational types of environments. The repeatability of the operational types of environments in space and time results in a common norm of variation for the individuals within and between populations.

The concept of "total" genetic variation of cultivated plants must be distinguished from variation in single population when the decision on sample size is based on one's mere experience. The very high sample size requirement for a population when based on the "total" genetic diversity of the species of crop, which is not defined and not known, may not always be desirable although it ensures the capture of rare alleles.

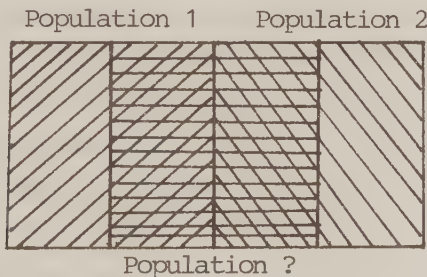
Hamrick et al. (1979), in comparisons of Lycopersicon esculentum and L. esculentum var. cerasiforme and Zea mays and Z. mexicana, showed that cultivated forms maintain more variation. On the other hand, they showed that non-cultivated Hordeum spontaneum and Phlox drummondii have considerable more variation than cultivated taxa (H. vulgare and P. drummondii). Thus, the level of variation maintained in a population does not always correspond to the high sample size requirement forecasted for cultivated forms.

The recommendations also suffer from overlooking the differences that exist in temperate, mediterranean, tropical and subtropical plant populations. Thus, Nevo (1978) found that habitat generalists and animal species with cosmopolitan or tropical distribution were typically more variable than species with specialized habitat preferences. This is biologically sound and to be expected. On the other hand, some plants in subarctic environment are found to have maintained unusually high genetic variation even at the climatic borderline of the species (Tigerstedt 1979). This could result from multiplicity of stress micro habitats in major climatic borderlines. There is another inherent and inescapable problem, when marginal and central populations appear to be in undifferentiated favourable or unfavourable heterogeneous environment. Under such conditions differences and/or similarities in the choice of sample size requirement between the two is not so obvious.

In the discussion of the sample size requirement the size

of the sample site is usually ignored. The bigger the sample site, the more variable point and the less random drift it has, unless the population is heterogeneous. Slopy, dissected terrains and primitive way of tilling overrule the size of a field. These fields are usually very small in all gene centers. Yearly differences in the size of site because of rotation of crops from one field to another within an area also complicate the decision on the sample size.

Another serious limitation of the various recommendations is the treatment of one region as a unit in order to sample more diverse regions (minimum sample number per region to maximize regional numbers) and also the underestimate of the differences of adjacent fields. If a field is not homogeneous (morphologically distinct sub-populations), then considering it as a unit of population amounts to mixing up the differences within the population. Thus, some farmers in Ethiopia grow local barley for flour, beer and roasted grain ("kollo") in separate patches but in the same field. Under such conditions there is a problem of sampling. Let us take two such populations with two alleles, A and a, at a locus.



Let $F(A)$ and $F(a)$ in population 1 be V_1 and u_1 respectively.

Let $F(A)$ and $F(a)$ in population 2 be V_2 and u_2 respectively. Let both population 1 and 2 be under Hardy-Weinberg equilibrium.

During the sampling of population ? a mistake is obviously made and the frequency of each class of gene type is:

	AA	Aa	aa
Observed	$\frac{1}{2}(V_1^2 + V_2^2)$	$V_1u_1 + V_2u_2$	$\frac{1}{2}(u_1^2 + u_2^2)$
Expected	$\frac{1}{2}(V_1 + V_2) \times \frac{1}{2}(V_1 + V_2)$	$\frac{1}{2}(V_1 + u_2)(V_2 + u_1)$	$1/4(u_1 + u_2)^2$

$F(Aa)$ observed is always less than $F(Aa)$ expected. The population is thus not taken to be in a Hardy-Weinberg equilibrium and there is a shortage of heterozygotes. One thus comes to a completely wrong conclusion since two different populations were sampled and considered as one population.

Collection of small samples from many populations in geographically differentiated regions forms good starting points, each field or fields being taken as a population or a subdivided population, unless free dispersal and high gene flow is assumed between the populations. Even this is subject to variation since the rate of outbreeding in any one region varies among localities and seasons, the levels of heterozygosity, rates of gene flow and scales of local differentiation all becoming probability statements, which force the investigator to take any

reasonable figure greater or less than the sample size recommended. Thus, Brown et al. (1978) have found 9.6% outcrossing in Hordeum spontaneum in some locations in Israel. At such a high gene flow a sample size corresponding to two or more adjacent populations is enough, but unfortunately it is not easy, if not impossible, to test a gene flow in the field, even where markers are present. Mitton and Pierce (1980) showed that the distribution of heterozygosity deviates from normality. This is a very important point, since decisions about sampling frequency, sample size etc. depend on certain assumptions concerning between and within population variances, levels of heterozygosity, and allelic frequencies at individual polymorphic loci. Statistical treatments here are based on normal distribution or some functions of it. Not only is the decision made on a wrong assumption but also prior to the necessary data.

Estimates of the parameters of the mating system are important in evolutionary genetics of both cultivated and wild biotypes. The present estimate of outcrossing rate was done mostly from 4 seeds/spike. Because of the association between the alleles of different loci (Brown et al. 1977), Brown et al. (1978) used a numerical multi-locus maximum likelihood procedure to estimate outcrossing. The observed number of heterozygotes per locus (H) and the outcrossing frequency (S) of the entire material studied is:

<u>Locus</u>	<u>H</u>	<u>S</u>
<u>Est-A</u>	28	0.0164
<u>Est-B</u>	0	0.0000
<u>Est-C</u>	20	0.0143
<u>Est-D</u>	0	0.0000
<u>Acph-1</u>	4	0.0059

From the above estimate the average outcrossing percentage is about 0.73%. But it varies from region to region as can be seen below from the observed number of heterozygotes.

<u>Region</u>	<u>No. of indi- viduals</u>	<u>Est-A</u>	<u>Est-B</u>	<u>Est-C</u>	<u>Est-D</u>	<u>Acph-1</u>
A	171	3	0	2	0	0
B	37	0	0	0	0	0
C	32	0	0	0	0	0
D	90	0	0	1	0	0
E	315	3	0	0	0	0
F	276	5	0	2	0	0
G	98	1	0	0	0	0
H	37	0	0	0	0	0
I	27	0	0	0	0	4
J	424	9	0	1	0	0
K	18	0	0	4	0	0
L	88	0	0	0	0	0
M	123	0	0	1	0	0
N	476	1	0	6	0	0
O	131	1	0	1	0	0
P	230	2	0	2	0	0
Q	266	1	0	0	0	0
R	315	1	0	0	0	0
S	112	1	0	0	0	0

The sample size requirement to detect heterozygosity or capture a rare allele, even in the same species, may not necessarily be the same in the same localities at different times. Such changes in gene frequency could result more by selection and in some cases by migration rather than by mutation.

It could even be argued that each population has its own requirement which can not, however, be fulfilled even when "information" on the population is available. Since genetic variation is a time series process the sample size worked out at one time is liable to change at another time. Alleles that were frequent at one time may become lost or at least change in their frequency. Such fluctuations of frequencies could be affected by seasonal variation, field rotation and environmental stress (like drought conditions). It is therefore unsafe to speak of constant sample size of any population at any time in any locality because of such various complications.

Populations of crops, in their seed base, could be represented from many generations by a farmer, adding the past genetic record to the new, retarding the decay of genetic variation of the past. But no study that shows the maintenance of genetic variation by this means is available, although this event perhaps would have been one of the contributory forces for genetic variation in crops. Effective numbers of alleles as a study tool are preferable here to frequencies of alleles and could be computed from frequencies of alleles by the formula $1/\sum x_i^2$, where x_i is the frequency of each allele.

Although it is generally accepted that alleles at polymorphic loci are used to forecast the sample size requirement there is the problem to decide on the number and type of loci to be considered. To consider the variation within some linked loci and various linkage groups could be useful, although according to Jain (1975) variation patterns like all other biological patterns are usually imperfect.

Crops are very vulnerable to drift since small samples selected by man in the form of few spikes from one region could be transferred to another region. History of the population and the tribes possessing it are worth noting. Sirkkomaa (1983) calculated the decrease of genetic variation because of the founder effect by considering some reasonable number of founders (2-50). He concluded that the expected loss of genetic variation at a two-allelic locus with unequal initial allele frequencies is generally larger for the number of alleles than for the allelic entropy (evenness). At equal initial allele frequencies the loss of the allelic entropy is larger than that for the number of alleles, but with more than 15 founders it is relatively insignificant, with small effect of the progeny population size on the expected loss, except for very unequal allele frequencies (Sirkkomaa 1983). From the study of Sirkkomaa's table (Sirkkomaa 1983, p. 18) 15-20 founders could represent the original base populations of barley presently studied in this project.

The best approach in all sampling methods would be to run a large number of individuals from many populations. But

resources do not always permit this. An approach in two steps should be taken. First find the pattern of alleles at low cost (for chemicals), then carry out a detailed study on a small number of populations. If we consider a major allele, it is the one that is present at $\geq 50\%$ (alleles that are usually conservationally important ones). To detect such an allele with at least 95% confidence ($1 - P^0_{qN} \geq 0.95$) requires not more than 5 individuals to be sampled from each population. If we calculate N for major alleles in each locus, as given in Table 4 of Bekele (1983a), we get the following values:

Locus	Allele	N
<u>Est-A</u>	<u>2</u>	2
<u>Est-B</u>	<u>6</u>	1
<u>Est-C</u>	<u>4</u>	2
<u>Est-D</u>	<u>3</u>	1
<u>Acph-1</u>	<u>2</u>	2

For safety, if ten times of N are to be taken, 20 spikes per population are needed. But only large samples will ensure the capture of a particular rare gene. It could be argued, however, that large sample size to capture rarest alleles from one population or region brings about the paradoxical result called the redundancy effect, large samples tending to include redundant copies of the same rare allele (Brown and Munday 1982). The probability (P)

of capturing at least one copy of an allele with population frequency p in a sample of n random gametes is:

$$P(p,n) = 1 - (1 - p)^n.$$

If an allele frequency is 0.001 and $n_1=100$ and $n_2=1000$ the probability of capturing $P(0.001,100)=0.095$ and $P(0.001, 1000)=0.632$ respectively. Thus, the total number of rare alleles captured by sampling 100 gametes from 10 populations exceeds the number captured by one large sample with $n=1000$ (Brown and Munday 1982). The sampling of rarest alleles could thus be recovered from the total number of individuals from the target area rather than its distribution between and within sampling sites. Considering allelic distributions between and within sampling sites, the sample size needed to detect true differences between the 19 regions studied could be estimated, based on the variability within each population by using Sokal's and Rohlf's (1969, p. 247) approach:

$$N \geq 2 \frac{\sigma^2}{\delta^2} \left[t_{\alpha}^2 v + t_{2(1-P)}^2 v \right]^2$$

where

N = desired sample size,

σ = true standard deviation (the square root of allelic variance,

δ = the smallest true difference that is desired to detect, e.g., 5%,

α = significance level of t ,

v = the number of degrees of freedom for the sample standard deviation,

P = desired probability that a difference will be found

to be significant, e.g. $P = 0.95$, and
 $t_{2(1-P), v}$ = values from a two-tailed t -table with v
 degrees of freedom and corresponding to probabilities
 of α and $2(1-P)$ respectively.

Theoretically, in a random sample of n gametes one would expect to capture

$$E(k) \approx \frac{\theta}{\theta} + \frac{\theta}{\theta+1} + \dots + \frac{\theta}{\theta+n-1}$$

neutral alleles, where $\theta = 4N_e u$ with N_e = the effective or breeding size of the population, and u = mutation rate per locus per generation (Ewens 1972).

According to Nei (1975, p. 131) the number of individuals to be studied per locus can be rather small (about 20 individuals). But differential breeding systems and population size in different species makes any suggestion of sample size questionable, since most recommendations are based on extrapolations from few investigated cases. The actual sample size detection from allelic frequencies and distributions should consider replication of sampling at intervals of time, and the fluctuations of environmental factors at these intervals of time. Any sampling policy for variation assessment and gene conservation activities must thus pay attention to the practical considerations inherent in such studies. Nevertheless, "true" representatives of populations would only appear from extensive investigations. An "ideal" magnitude of sample and patterns of variation within a species could only be estimated from a collection of populations representing the whole range of distribution and habitat sampled in

replications at time intervals.

Sampling problems for gene conservation studied in this project could be put in the following versions:

Version 1

In an infinite population individuals are of one of the types $G_1, G_2, G_3, \dots, G_A$; these types occur with frequencies $p_1, p_2, p_3, \dots, p_A$. What sample size, n , will give any G_a occurring with a frequency that is not too small, say $p_a \geq p_0$, a comfortably large chance, say at least $1 - \alpha$, of appearing at least once in a simple random sample of size n ? Of course n is a function of p_0 and α : $n = n(p_0, \alpha)$.

Here we look at one genotype at a time. If G_j has $p_j \geq p_0$, the probability that G_j is absent in a sample of size n is

$$(1 - p_j)^n$$

and that probability is at most $(1 - p_0)^n$.

Hence our task is to determine n in such a way that $(1 - p_0)^n \leq \alpha$, where α is the risk we are willing to take of missing a genotype whose frequency is at least p_0 . Solving the equation

$$(1 - p_0)^n = \alpha$$

gives $n = \log \alpha / \log(1 - p_0)$.

Version 2

In an infinite population where the different types occur with frequencies $p_1, p_2, p_3, \dots, p_A$, what sample size, n , will make the chance at least $1 - \alpha$ that every G_a with $p_a \geq p_0$ is represented at least once in a simple random

sample of size n ?

Here if we let our sample size be the smallest n for which $Kp_0 \leq 1$, where K is the number of genotypes in a population,

$$G_n(K, p_0) = K(1-p_0)^n - \binom{K}{2}(1-2p_0)^n + \binom{K}{3}(1-3p_0)^n + \dots$$

$$\dots + (-1)^{K+1}(1-Kp_0)^n \leq \alpha$$

$$= \sum_{j=1}^K (-1)^{j-1} \binom{K}{j} (1-jp_0)^n \leq \alpha$$

The simplest and more conservative approximation is obtained by skipping all terms except the first one in the above expression, giving the inequality

$$K(1-p_0)^n \leq \alpha$$

$$n = \log(\alpha/K) / \log(1-p_0)$$

Version 3

Every population can be described by the type frequencies $p_1, p_2, p_3, \dots, p_A$; these, however, vary from population to population. If we imagine the populations to be drawn from some (possibly infinite) population of populations, can we then describe how these frequencies $p_1, p_2, \dots, p_A$, vary between the different populations and can we assert how often it will happen that a type G_a , which is present in a certain population, will be missing in a simple random sample of size n from that population?

Here only results of version 1 and 2 will be presented since version 3 and possibly other versions to be studied demand a model construction which is in progress.

Assuming that the entire sample is one finite population and using weighted frequency of each allele in each locus the sample size n that will give an allele with P_0 observed frequency at $\alpha = 0.10$ could be solved by

$$n = \log \alpha / \log(1 - P_0)$$

as shown before in version 1.

From Table 1, it is obvious that when one uses the entire material as one big population with $N=3266$, all the alleles except Est-A-3 and Acph-1-6 could be captured at $\alpha = 0.10$.

In version 2, where we considered a simultaneous statement on all genotypes ($= K$), the N values for

$$K = 3, 4, 5, 6, 7, 8, 9, 10$$

$$P_0 = 0.10, 0.05, 0.01$$

$$\alpha = 0.10, 0.05, 0.01$$

are given in Table 2a, b and c.

When one compares the N values of Table 2 with the actual N values in the 19 regions studied, the sample size taken per region is appropriate for $P_0 = 0.10$ and $P_0 = 0.05$ in most cases.

Besides the problems of sample size discussed above, frequencies of alleles and genotypes in the generation of

Table 1. The sample size n that will give an allele with P_0 observed frequency at $\alpha = 0.10$.

Locus	Allele	P_0	n
<u>Est-A</u>	0	0.0067	345
	1	0.3860	5
	2	0.6039	3
	3	0.0007	3 333
	4	0.0031	769
<u>Est-B</u>	0	0.0616	36
	5	0.0016	1 429
	6	0.9289	1
	7	0.0082	278
<u>Est-C</u>	0	0.0009	2 500
	2	0.0079	294
	3	0.2827	7
	4	0.6656	2
	5	0.0409	55
	6	0.0018	1 250
<u>Est-D</u>	0	0.0228	170
	2	0.0463	49
	3	0.8741	1
	4	0.0578	39
	5	0.0083	278
<u>Acph-1</u>	0	0.0228	100
	1	0.1501	14
	2	0.6568	2
	3	0.1632	13
	4	0.0061	370
	6	0.0006	3 333

Table 2. Required sample size, N , at different values of K , p_0 and α .

a) $p_0 = 0.10$

K	α		
	0.10	0.05	0.01
3	33	39	55
4	35	42	57
5	37	44	59
6	39	46	61
7	41	47	63
8	42	49	64
9	43	50	65
10	44	51	66

b) $p_0 = 0.05$

K	α		
	0.10	0.05	0.01
3	66	80	112
4	72	86	117
5	76	90	122
6	80	94	125
7	83	97	128
8	85	99	131
9	88	101	133
10	90	103	135

c) $p_0 = 0.01$

K	α		
	0.10	0.05	0.01
3	336	406	568
4	364	435	596
5	386	457	618
6	404	475	637
7	419	490	652
8	432	503	665
9	444	515	677
10	454	526	687

mature plants collected (generation n) in relation to the generation of rejuvenated plants (generation $n + 1$) may be a problem because of genetic recombination. However, since all the crops being dealt with here are predominantly self-fertilizing, recombination will be low, most seedlings having the same genotypes as the maternal plant. From the viewpoint of population dynamics of genes, selfing is similar to asexual reproduction (Nei 1975, p. 143). Although all gametes are produced through meiotic division selfing prohibits the recombination of mutant genes that occur in different individuals. Just as in asexual organisms, the whole set of genes in an individual is transmitted together to the offspring, though at a small proportion of loci gene segregation would occur because of occasional mutations (Nei 1975, p. 143). In self-fertilizing plants gametes are generally less well mixed in the process of reproduction than gametes in outbreeding organisms, and thus the effective population size appears to be relatively small.

In addition to the above problems of gene erosion due to sampling, differential survival at storage in the gene bank, chromosomal aberrations and gene mutations during seed storage (Roberts 1973, 1975, 1978) successively contribute, not only to a simple gene erosion, but also to the backgrounds of the gene (genome erosion). The splitting of one population into several parts in order to facilitate the needed immediate evaluation of the samples collected does not guarantee the preservation of rare alleles or rare gene complexes. The more useful approach is to keep each individual of the population alone.

Unlike the adopted gene bank way of keeping the whole population as a bulk, partitioning the populations into individuals and keeping individual genotypes of populations separately, will guarantee the preservation of rarer alleles and makes it selfamenable to the study of population structure. This will make evaluated individuals of populations easily tracked. It also makes it easier to estimate the behaviour of a population in its genetic changes during the period of storage. Individuals of minimum genetic change could be looked at as part of the population's characteristics. The immediate utility of such characteristics might turn out to be economically important, since there may well be differential genetic changes and only those genotypes that need frequent rejuvenation have to be taken into consideration.

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Literature cited

- Adary, A.H. 1978. Genetic variation in land races of durum wheat and its value in durum wheat improvement. - Ph.D. Dissertation, Univ. of California, Davis, USA
- Allard, R.W. 1970. Population structure and sampling methods. - In Genetic Resources in Plants, their Exploration and Conservation (Eds. O.H. Frankel and E. Bennett), Blackwell, Oxford, p. 97-107
- Allard, R.W., Harding, J. and Wherhahn, C. 1966. The estimation and use of selective values in predicting population change. - Heredity 21: 547-563
- Almgard, G. and Landegren, U. 1974. Isoenzymatic variation used for the identification of barley cultivars. - Z. Pflanzenzücht. 72: 63-73
- Barton, K.A. and Brill, W.J. 1983. Prospects in plant genetic engineering. - Science 219: 671-676
- Bennett, E. 1965. Plant introduction and genetic conservation; genecological aspects of an urgent world problem. - Scottish Plant Breed. Station Rec., p. 27-113
- Bennett, E. (Ed.) 1968. FAO/IBP Technical Conference on the exploration, utilization and conservation of plant genetic resources. - Rome, Italy
- Bennett, E. 1970. Tactics of plant exploration. - In Genetic Resources in Plants, their Exploration and Conservation (Eds. O.H. Frankel and E. Bennett), F.A. Davis, Philadelphia, p. 157-188
- Borlaug, N.E. 1983. Contribution of conventional plant breeding to food production. - Science 219: 689-693
- Brown, A.H.D. 1978. Isozymes, plant population genetic structure and genetic conservation. - Theor. Appl. Genet. 52: 145-157

- Brown, A.H.D. 1983. Barley. - In Isozymes in Plant Genetics and Breeding. Part B (Eds. S.D. Tanksley and T.J. Orton), Elsevier Science Publishers, B.V., Amsterdam, p. 57-77
- Brown, A.H.D. and Clegg, M. 1983. Isozyme assessment of plant genetic resources. - In Isozymes: Current Topics in Biological and Medical Research (Eds. M.C. Rattazzi, J.G. Scandalios and G.S. Whitt), Alan R. Liss, Inc., New York, p. 285-295
- Brown, A.H.D., and Munday, J. 1982. Population-genetic structure and optimal sampling of land races of barley from Iran. - Genetica 58: 85-96
- Brown, A.H.D., Nevo, E. and Zohary, D. 1977. Association of alleles from esterase loci in wild barley, Hordeum spontaneum. - Nature 268: 430-431
- Brown, A.H.D., Zohary, D. and Nevo, E. 1978. Outcrossing rates and heterozygosity in natural populations of Hordeum spontaneum Kock in Israel. - Heredity 41: 49-62
- Cavalli-Sforza, L. 1978. Replacing nature as innovator. - In Genetic Engineering (Eds. H.W. Boyer and S. Nicosia), Elsevier/North Holland, p. 3-8
- Cavalli-Sforza, L. and Bodmer, W.F. 1971. The Genetics of Human Populations. - W.H. Freeman and Co., San Fransisco
- Chaleff, R.S. 1983. Isolation of agronomically useful mutants from plant cell cultures. - Science 219: 676-682
- Clausen, J. and Hiesey, W.M. 1958. Experimental studies on the nature of species. IV. Genetic structure of ecological rates. - Carnegie Inst. Wash. Publ. No. 615
- Conrad, M. 1983. Adaptability. The Significance of Variability from Molecule to Ecosystem. - Plenum Press, New York
- Crow, J.F. and Kimura, M. 1970. An Introduction to Population Genetics Theory. - Harper Int. Ed., Harper and Row Publ. New York

- Darlington, C.D. 1969. The silent millenia in the origin of agriculture. - In The Domestications and exploitation of plants and animals (Eds. P.J. Ucko and G.W. Dimbleby). - Duckworth, London, p. 67-72
- De Candolle, A. 1959. Origin of Cultivated Plants. 2nd ed. - Kegan Paul, London
- Denniston, C.D. 1978. Small population size and genetic diversity: Implications for endangered species. - In Endangered Birds: Management Techniques for Preserving Threatened Species (Ed. S.A. Temple), University of Wisconsin Press, Madison, U S A, p. 281-289
- Dionne, L.A. 1961. Mechanism of interspecific incompatibility in tuber bearing Solanum species. - Am. Potato J. 38: 73-77
- Dionne, L.A. 1963. Studies on the use of Solanum acaule as a bridge between Solanum tuberosum and species in the series Bulbocastana, Cardiophylla and Pinnatisecta. - Euphytica 12: 263-269
- Dorofeev, V.F. and Jakubziner, M.M. 1973. The gene pool of Soviet durum wheat (Triticum durum Desf). - Proc. Int. Symp. on Genetics and breeding of durum wheat, Univ. of Bari, Italy, p. 141-152
- Ewens, W.J. 1972. The sampling theory of selectively neutral alleles. - Theor. Pop. Biol. 3: 87-112
- Flor, H.H. 1955. Host-parasite interaction in Flax-rust - its genetics and other implications. - Phytopathology 45: 680-685
- Ford, E.B. 1975. Ecological Genetics (4 ed.). - Chapman and Hall, London
- Fox, S.F. 1975. Natural selection on morphological phenotypes of the lizard, Uta stansburiana. - Evolution 29: 95-107

- Frankel, O.H. 1972. The significance, utilization and conservation of crop genetic resources. - FAO, Rome
- Frankel, O.H. and Bennett, E.(Eds). 1970. Genetic Resources in Plants - Their Exploration and Conservation. - Int. Biol. Prog. II, Blackwell Scientific Publ., Oxford
- Frankel, O.H. and Hawkes, J.G. 1975. Genetic Resources - the past ten years and the next. - In Crop Genetic Resources for Today and Tomorrow (Eds. O.H. Frankel and J.G. Hawkes), Int. Biol. Prog. II, Cambridge Univ. Press, p. 1-11
- Frankel, O.H. and Soulé, M. 1981. Conservation and Evolution. - Cambridge Univ. Press
- Franklin, I.A. 1980. Evolutionary change in small populations. - In Conservation Biology: An Evolutionary Ecological Perspective (Eds. M.E. Soulé and B.A. Wilcox), Sinauer Associates, Sunderland, Mass., p. 135-150
- Griffing, B. 1976a. Selection in reference to biological groups. V. Analysis of full-size groups. - Genetics 82: 703-722
- Griffing, B. 1976b. Selection in reference to biological groups. VI. Use of extreme forms of non-random groups to enhance selection efficiency. - Genetics 82: 723-731
- Gustafsson, Å. 1953. The cooperation of genotypes in barley. - Hereditas 39: 1-18
- Hamrick, J.L., Linhart, Y.B. and Mitton, J.B. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. - Ann. Rev. Ecol. Syst. 10: 173-200
- Harlan, J.R. 1951. Anatomy of gene centers. - Am. Nat. 85: 97-108
- Harlan, J.R. 1971. Agricultural origin: Centers and non-centers. - Science 174: 468-474

- Harlan J.R. 1975. Crops and Man. - Am. Soc. Agron., Madison, Wisconsin
- Harper, J.L. 1965. The nature and consequences of interference among plants. - In Genetics Today. Proc. XI. Int. Congr. Genet. Sept. 2-10, 1963, 2. Pergamon Press, Oxford, p. 465-482
- Hawkes, J.G. 1983. The diversity of crop plants.- Harvard Univ. Press, Cambridge, Mass.
- Hui-Lin, Li. 1970. The origin of cultivated plants in South-East Asia. - Econ. Bot. 24: 3-19
- Jain, S.K. 1975. Genetic reserves. - In Crop Genetic Resources for Today and Tomorrow (Eds. O.H. Frankel and J.G. Hawkes), IBP2, Cambridge University Press, p. 379-396
- Jinks, J.L. 1978. Personal communication. - Univ. of Birmingham, U.K. M.Sc. lecture note
- Kahler, A.L. and Allard, R.W. 1981. World wide patterns of genetic variation among four esterase loci in barley (Hordeum vulgare L.). - Theor. Appl. Genet. 59: 101-111
- Karlin, S. and Mc Gregor, J. 1974. Towards a theory of evolution of modifier genes. - Theor. Pop. Biol. 5: 59-103
- Legendre, P. 1971. Some formal aspects of the theory of biological evolution. - PhD thesis, Univ. of Colorado
- Leppik, E. 1968. Relation of centres of origin of cultivated plants to sources of disease resistance. - Plant Introd. Investigation Paper, no. 13, - Beltsville, Maryland, p. 7
- Marshall, D.R. and Brown, A.H.D. 1975. Optimum sampling strategies in genetic conservation - In Crop Genetic Resources for Today and Tomorrow (Eds. D.H. Frankel and J.G. Hawkes), IBP2, Cambridge University Press, p. 53-80

- Mather, K. 1961. Competition and Cooperation. - Symp. Soc. Exp. Biol. 15: 264-281
- Mayr, E. 1969. Populations, Species and Evolution. - The Belknap Press of Harvard Univ. Press, Cambridge, Mass.
- Mayr, E. 1982. The Growth of Biological Thought. - The Belknap Press of Harvard University Press, Cambridge, Mass.
- Merker, A. 1984. The rye genome in wheat breeding. - Hereditas 100: 183-191
- Minoru, M. 1979. Genetic changes induced by artificial seed aging in barley. - PhD thesis, Colorado State University
- Mitton, J.B. and Pierce, B.A. 1980. The distribution of individual heterozygosity in natural populations. - Genetics 95: 1043-1054
- Müntzing, A. 1966. On the evolution and breeding of cultivated plants. - Indian J. Genet. 26: 3-13
- Muona, O.A. 1980. Evolution of correlated genetic systems in barley: Morphological and enzyme polymorphisms, quantitative characters and disease. - PhD Thesis, Univ. of California, Davis, U.S.A.
- Nei, M. 1967. Modification of linkage intensity by natural selection. - Genetics 57: 625-641
- Nei, M. 1973. Analysis of gene diversity in subdivided populations. - Proc. Natl. Acad. Sci. USA. 70: 3321-3323
- Nei, M. 1975. Molecular Population Genetics and Evolution. - North Holland, Amsterdam
- Nei, M., Maruyama, T. and Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. - Evolution 29: 1-10
- Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. - Theor. Pop. Biol. 13: 121-177

- Nielsen, G. and Frydenberg, O. 1972. Distribution of esterase isozymes, α -amylase types and DDT reactions in some European barleys. - Z. Pflanzenzücht. 68: 213-224
- Qualset, C.O. 1975. Sampling germplasm in a center of diversity: an example of disease resistance in Ethiopian barley. - In Crop Genetic Resources for Today and Tomorrow (Eds. O.H. Frankel and J.G. Hawkes), IBP2, Cambridge Univ. Press. p. 81-96
- Riedl, R. 1978. Order in Living Organisms. - John Wiley and Sons, Chichester, N.Y.
- Roberts, E.H. 1973. Loss of viability: Chromosomal and genetical aspects. - Seed Sci. Technol. 1: 515-527
- Roberts, E.H. 1975. Problems of long-term storage of seeds and pollen for genetic resources conservation. - In Crop Genetic Resources for Today and Tomorrow (Eds. O.H. Frankel and J.G. Hawkes), Int. Biol Prog. 2, Cambridge Univ. Press, London, p. 269-296
- Roberts, E.H. 1978. Mutations during seed storage. - Acta Hortic. 83: 279-282
- Sakai, K.J. 1955. Competition in plants and its relation to selection. - Cold Spring Harbor Symp. Quant. Biol. 20: 137-157
- Sandfaer, J. 1970. An analysis of the competition between some barley varieties. - Danish Atomic Comm. Res. Establ. Risø Report no. 230
- Sandfaer, J. 1977. Effects of a virus on competition and selection in barley. - In Measuring Selection in Natural Populations (Eds. F.B. Christiansen and T.M. Fenchel), Lecture Notes in Biomathematics, Vol. 19, Springer, Heidelberg, p. 465-475
- Shepard, J.F., Bidney, D., Barsby, T. and Kemble, R. 1983. Genetic transfer in plants through interspecific protoplast fusion. - Science 219: 683-688

- Sirkkomaa, S. 1983. Calculations on the decrease of genetic variation due to the founder effect. - Hereditas 99: 11-20
- Sokal, R.R., and Rohlf, F.J. 1969. Biometry.- W.H. Freeman and Co., San Francisco
- Steel, R.G.B. and Torrie, J.H. 1960. Principles and procedures in statistics. - Mc Graw-Hill, New York
- Tigerstedt, P.M.A. 1979. Genetic adaptation of plants in the subarctic environment. - Holarctic Ecol. 2: 264-268
- Turesson, G. 1922. The genotypical response of the plant species to the habitat. - Hereditas 3: 211-350
- U.S. Dept. of Agriculture Nov. 1973. Recommended actions and policies for minimizing the genetic vulnerability of our major crops. - The U.S. Department of Agriculture
- Walker, J.T. 1969. Selection and quantitative characters in field crops. - Biol. Rev. 44: 207-243
- Wiebe, G.A., Peter, F.C. and Stevens, H. 1963. Interplant competition between barley genotypes. Statistical genetics and plant breeding. - In Symp. Raleigh, North Carolina, March 20-29, 1961. Natl. Acad. Sci. - Natl. Res. Counc. 982: Washington D.C., p. 546-555
- Williams, E.J. 1962. The analysis of competition experiments. - Aust. J. Biol. Sci. 15: 509-525
- Willis, C. 1981. Genetic Variability. - Clarendon Press, Oxford
- Wright, S. 1931. Evolution in Mendelian populations. - Genetics 16: 97-159
- Wright, S. 1943. Isolation by distance. - Genetics 28: 114-138
- Wright, S. 1970. Random drift and the shifting balance theory of evolution. - In Mathematical Topics in Population Genetics (Ed. K. Kojima), Springer, Berlin, p. 1-31

- Zemed, A. 1980. An ecophysiological study of cultivated barleys in Welmera. - M.Sc. thesis presented to the school of graduate studies, Addis Abeba Univ., Ethiopia
- Zhukovsky, P.M. 1969. Mega-gene Centers and Micro-gene Centers. - In N.I. Vavilov and Agricultural Science 120-202. Moscow

